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EFFECT OF MYCOTOXINS DAS, ZEA AND OTA ON THE GROWTH OF BREWING YEAST

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Key words: mycotoxins, brewing yeasts, yeast growth

The aim of the study was to assess how different concentrations of diacetoxyscirpenol (DAS), zearalenone (ZEA) and ochratoxin A (OTA) affect the growth of 4 brewing yeast strains in the model medium YEPG. Determinations were carried out for the following parameters: specific growth rate, duration of the log phase, and biomass yield. The results of the study showed that OTA exerted no toxic effect over the concentration range of 2.5 to 50 μg/mL. ZEA had an adverse influence on yeast growth only following application of the highest doses (50 and 100 μg/mL). DAS showed the highest toxicity, which inhibited the growth of all strains examined, even at the lowest concentration (2.5 μg/mL) applied. Each of the brewing yeasts strains showed a specific sensitivity to the toxins. The strains *S. carlsbergensis 13* and *S. cerevisiae 46* were found to be the least resistance, particularly to DAS.

INTRODUCTION

The presence of epiphytic microflora on cereal grains is a potential contributory factor in the formation of mycotoxins. The growth of toxinogenic fungi becomes particularly intense during a wet vegetative season or when the grain was treated inadequately after the harvest. Of the microorganisms generating in-field infections, *Fusarium ssp.* poses the most severe threat to the crops. The numerous species, *e.g. F. graminearum, F. culmorum, F. moniliforme, F. graminis, F. sporotrichoides*, produce zearalenone (ZEA) and mycotoxins of the trichothecene group: deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2 toxin and verrucarin [Gutleb *et al*., 2002]. The compounds are detected notably in wheat, rye, barley and maize grains [Perkowski *et al*., 2003; Müller *et al*., 1997; Tanaka *et al*., 1988]. Inappropriate storage of the grain induces the growth of *Aspergillus flavus* and *Aspergillus parasiticus* (responsible for the synthesis of aflatoxins), *Aspergillus ochraceus*, and some species of the genus *Penicillia* (those producing ochratoxins) [Chełkowski *et al*., 1979; Flanningan, 1987].

Brewing barley with visible symptoms of mould growth is not processed for obtaining malt. Nevertheless, it is essential to note that, being natural components of the microflora of barley, mycotoxin-producing fungi gain the potentiality for the development and synthesis of toxins during the malting process. There is ample evidence, for example, that malt obtained from grain naturally infected with *Fusarium graminearum* contained deoxynivalenol (DON) at the level of 18 to 114% of the initial value. Moreover, germination was found to increase the amounts of 15-acetyldeoxynivalenol and zearalenone [Schwarz *et al*., 1995].

Mycotoxins can enter the technological process together with malt or with a non-malted substrate (*e.g.* maize). Generally, the quantity of mycotoxins is notably reduced at particular stages of beer production. Some part of toxins reduces in the mashing process, some part persists in spent grain while some part undergoes transformation during wort hopping [Baxter *et al.*, 2001; Flanningan, 1987]. Some of the toxins, *e.g.* DON and T-2, display a high stability; they are able to survive thermal treatment and enter the final product [Perkowski, 2000; Schwarz *et al*., 1995]. The transmission of mycotoxins into the wort poses a threat to the fermentation process, as these compounds can exert an adverse effect on the activity of yeasts and thus disturb, for example, the process of biomass proliferation.

The primary objective of the study was to determine the influence of different concentrations of the mycotoxins DAS, ZEA and OTA on the course and efficiency of brewing yeast growth in the model medium YEPG. Another major objective was to establish the toxin doses with which the inhibition of yeast growth is a moderate one. The results obtained will be used at a further stage of the study where fermentation tests will be performed with contaminated worts.

MATERIALS AND METHODS

The experimental material used in this study included mycotoxins purchased from Sigma Aldrich Sp.z o.o.: diacetoxyscirpenol (DAS), ochratoxin A (OTA) and zearalenone (ZEA).The biological material consisted of the following yeast strains: *Saccharomyces carlsbergensis* I-S.ca./13, *Saccharomyces cerevisiae* (lager) 23, *Saccharomyces cerevisiae* I-S.c./46 and *Saccharomyces cerevisiae* I-S.c./57.

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The strains S.ca. 13, S.c. 46 and S.c. 57 came from the collection of the Institute of Agricultural and Food Biotechnology, Warsaw. The strain S.c. 23 was isolated at the Department of Food Storage and Technology, Wrocław University of Environmental and Life Sciences, from a 48-hour culture of dry brewing yeasts Saflager S-23 (Fermentis Division of S.I.Lesaffre, France).

The yeasts were grown in a YEPG medium composed of 2% glucose, 1% yeast extract and 1% peptone (pH 5).

The study aimed at assessing the ability of yeasts to grow in presence of one of the three toxins over concentration range of 2.5, 5, 10, 15 and 20 μg/mL growth medium. Additionally, the medium was contaminated with ZEA doses of 50 and 100 μg/mL and OTA dose of 50 μg/mL. The toxins were added into the medium in the form of ethanol solution. The control was a yeast culture with no toxin, but with an equivalent amount of ethanol that was used in the contaminated media.

Cultivation was conducted in the turbidimeter Bioscreen C (Labsystem, Finland). Samples of contaminated medium (each of a 300 μL volume) were placed in cells of the Bioscreen C plates. Both the uncontaminated and mycotoxin-contaminated media were inoculated with 50 μL of yeast inoculum of a concentration amounting to 10^6 cfu/mL. Cultivation was conducted for 96 h with the following parameters: temperature, 25°C; average degree of mixing, every 10 s; automatic measurement of optical density (OD), every 20 min.

Using results of OD measurements, yeast growth curves were plotted and the following parameters were computed: specific rate of biomass growth (μ) in the log phase, duration of the log phase (as the difference between the hour when the stationary phase commenced and the length of the lag phase), and the maximal biomass yield (ΔOD_{max}). The results of research were presented in Figures 1-3 and Tables 1-3 as average values of three repetitions.

TABLE 1. Specific growth rate (h^{-1}) of brewing yeasts in the YEPG medium contaminated with DAS, ZEA and OTA.

Toxin	Toxin concentration (μg/mL)	Yeast strain			
		S.ca.13	S.c.23	S.c.46	S.c.57
Control	0	0.152	0.122	0.164	0.123
DAS	2.5	0.109	0.118	0.109	0.099
	5	0.046	0.102	0.049	0.074
	10	0.027	0.088	0.021	0.054
	15	0.022	0.087	0.028	0.061
	20	0.030	0.069	0.030	0.043
ZEA	2.5	0.147	0.120	0.151	0.125
	5	0.152	0.122	0.157	0.127
	10	0.153	0.118	0.153	0.125
	15	0.147	0.119	0.142	0.128
	20	0.138	0.120	0.137	0.132
	50	0.032	0.074	0.039	0.063
	100	0.032	0.059	0.062	0.060
OTA	2.5	0.153	0.116	0.161	0.126
	5	0.146	0.121	0.156	0.125
	10	0.152	0.115	0.151	0.123
	15	0.140	0.118	0.147	0.121
	20	0.146	0.114	0.158	0.120
	50	0.148	0.119	0.158	0.115

RESULTS

The tests with control samples have revealed differences in the specific growth rate between particular brewing yeast strains (Table 1). The highest growth rates were obtained for S.ca. 13 (0.15 h^{-1}) and S.c. 46 (0.16 h^{-1}). The other strains, S.c. 23 and S.c. 57, showed the same growth rate (0.12 h^{-1}) in the uncontaminated medium.

Even with the lowest dose applied (2.5 μg/mL), contamination of the medium with diacetoxyscirpenol had an inhibiting effect on the growth of brewing yeasts (Figure 1). As the DAS dose increased, the rate of biomass growth decreased continually but the extent of those changes varied from one yeast strain to another (Table 1). DAS exerted the strongest inhibiting effect on growth of the fast growing strains S.ca. 13 and S.c. 46. The yeast strain S.c. 23 was found to be the most immune to presence of DAS in the medium. Compared to other strains, decrease in the growth rate of S.c. 23 was the smallest even with the highest DAS dose applied (20 μg/mL).

In the presence of zearalenone up to 20 μg/mL, the brewing yeasts followed a growth pattern similar to that of the uncontaminated samples (Figure 2). Application of two higher doses, 50 and 100 μg/mL, brought about a distinct inhibition of biomass cultivation, which manifests in low values of specific growth rate (Table 1). Contamination of medium with these two ZEA doses accounted primarily for delay in the growth of strains S.ca.13 and S.c. 46, which practically began to proliferate on the second day of cultivation.

In contrast to DAS and ZEA, ochratoxin A did not inhibit the growth of brewing yeasts studied (Figure 3). Irrespective of OTA dose, their growth rates as well as the length of log phase were comparable to those of the control samples (Tables 1 & 2).

As for DAS and ZEA, the growth rate pattern described above refers to the phase of logarithmic growth, and its duration depends on the toxin dose added to the medium (Table 2).

The increase of DAS concentration in medium extended duration of the log phase period, which is attributable to reduced rate of the biomass growth. This finding holds true particularly for strains S.ca. 13 and S.c. 46; in their presence, duration of the log phase was extended to over 50 h at a concentration of DAS 15 μg/mL (Table 2). With a higher dose, 20 μg/mL, the log phase of S.ca. 13 and S.c. 46 growth was shorter but the biomass yield under such conditions was very poor.

If the dose applied did not exceed 20 μg/mL, the presence of ZEA had no significant effect on duration of the log phase of yeast growth (Table 2). Application of 50 μg/mL dose extended length of the log phase by 8 h on average. With the highest ZEA dose, duration of the log phase was also long but its length varied between 7 and 20 hrs. It should be noted, however, that the strains S.c. 23 and S.c. 57 began to proliferate at an exponential rate in the first hour of cultivation while the strains S.ca. 13 and S.c. 46 needed approximately 30 and 17 h, respectively, to adapt to the contaminated environment. The time-lag of the phase of logarithmic growth, combined

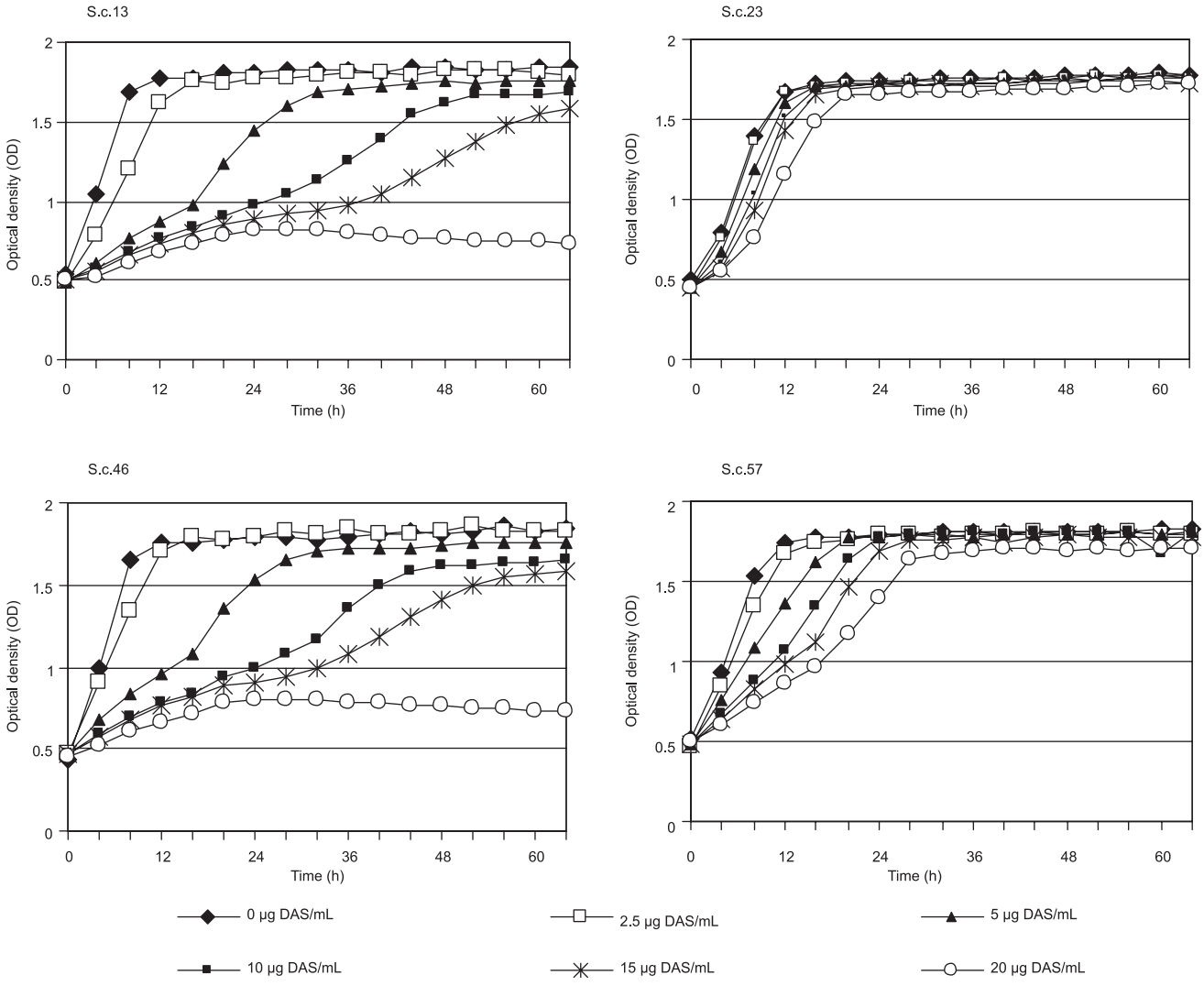

FIGURE 1. Growth curves of brewing yeasts in YEPG medium contaminated with DAS.

with the low specific growth rate of these yeast strains, resulted in a low biomass yield.

The quantity of biomass produced resulted from combined effect of the growth rate and the length of the log phase. As was the case with parameters described above, the presence of OTA in medium had no influence on the biomass yield (Table 3).

The response of brewing yeasts to occurrence of DAS in medium varied according to the strain used. Yeasts S.c. 23 and S.c. 57 were found to have the best resistance response to presence of this toxin, and final biomass yield was comparable with that of the control (Table 3). The increase in concentration of DAS was concomitant with a decrease of biomass yield in the case of S.c. 13 and S.c. 46. With the highest dose, 20 μg/mL, the biomass yield obtained amounted to only 20% of the biomass quantity produced in the uncontaminated samples.

The biomass in the ZEA-contaminated samples showed a reduced yield only with application of the highest doses: 50 and 100 μg/mL. There is, however, one exception worth mentioning: the strain S.c. 23, which produced (at the highest ZEA dose) a biomass quantity only by 7% lower as compared to the control.

DISCUSSION

The quantity and good condition of yeasts at the end of fermentation that enables their reuse is a matter of great importance in brewing industry, since the two factors influence fermentation rate and time required for termination of the process. The condition of yeasts depends on the composition of wort – specifically on such components as sugars, nitrogen compounds, mineral matter and vitamins. It may occur, however, that some compounds generally regarded as being harmful to yeasts penetrate into the wort from the substrate. Such are, *e.g.*, the mycotoxins produced by *Fusarium, Aspergillus* or *Penicillium ssp*.

The aim of the study reported on in this paper was to examine the influence of three toxins of choice, diacetoxyscirpenol (DAS), ochratoxin A (OTA) and zearalenone (ZEA), on the growth of different brewing yeast strains. The results of our study have confirmed observations made by other investigators, who have demonstrated – taking *Fusarium* mycotoxins (ZEA, FB_1, DON, NIV) as examples – that these metabolites affect the growth of lager and ales strains, and that the inhibiting effect depends on toxin type and con-

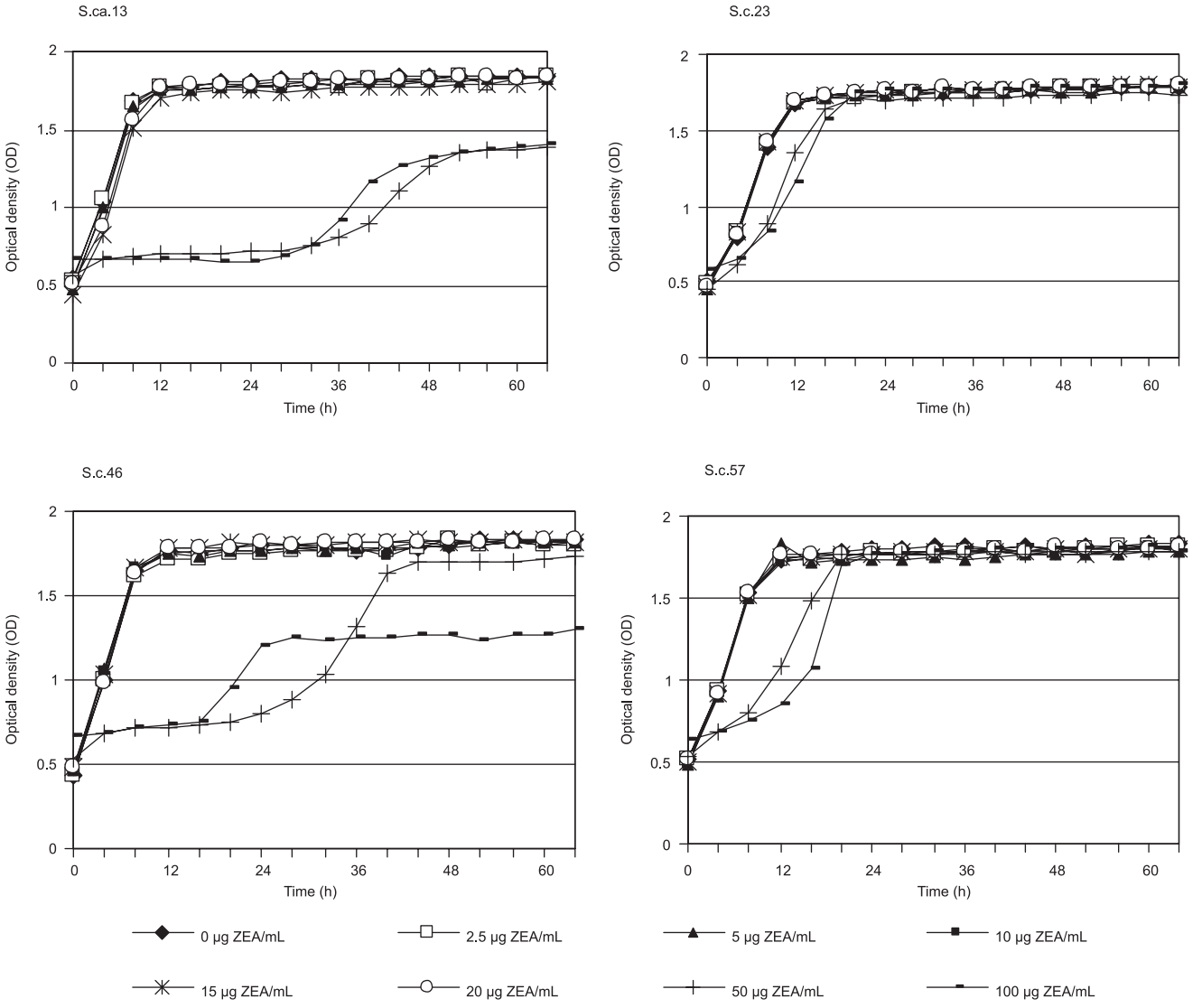

FIGURE 2. Growth curves of brewing yeasts in YEPG medium contaminated with ZEA.

centration, yeast strain, length and temperature of incubation, and method used for growth examination [Boeira *et al*., 1999a,b; 2002].

Of the mycotoxins used, diacetoxyscirpenol was found to be the strongest inhibitor of yeast growth; in the presence of DAS, the biomass proliferation was already inhibited with the lowest dose applied, *i.e.* 2.5 μg/mL. A similar inhibiting effect due to DAS has been reported by Whitehead & Flanningan [1989]: at the concentration of 5 μg/mL the number of *S. cerevisiae* cells was reduced by approximately 55%; the increase of DAS dose to 10 μg/mL accounted for cell reduction of about 62%. DAS belongs to the trichothecenes that are recognized as inhibitors of protein synthesis in sphaeroplasts of *S. cerevisiae* [Boeira *et al*., 1999b]. Another toxin from this group, T-2 toxin inhibits oxygen consumption of yeast cell and disturbs mitochondrial function at the level of the electron transport chain [Koshinsky *et al*., 1988]. Similar to DAS, T-2 toxin strongly influences yeast growth [Boeira *et al*., 2000; Foszczyńska *et al*., 2006; Schappert & Khachatourians, 1983].

Zearalenone is a toxin of a substantially weaker impact. In our studies, the yeasts developed a response to the presence of ZEA only at the highest concentrations, 50 and 100 μg/mL that is comparable to findings reported by Boeira *et al*. [1999a]. As for ochratoxin A, we found that OTA did not affect the yeast cells even at a very high dose – 50 μg/mL. This indicates that the toxicity of OTA was low or that the yeasts have adapted to the presence of the contaminant. As yet, this problem has not been sufficiently considered in the available literature.

The brewing yeast strains differed in their sensitivity to the toxins. It could be related with an ability of toxin to penetrate through the yeasts cell membrane. Boeira *et al*. [2000] have examined 5 yeast genera for their resistance to various toxins. The results indicated that *Kluyveromyces marxianus* and *Schisaccharomyces pombe* were the most sensitive, especially to the toxin T-2. In author's opinion, this may in part result from differences in the structural composition of the plasma membrane and therefore uptake of toxin by yeast cells. An additional factor limiting the penetration of toxins into the cell interior is the possibility of binding them by the cell wall.

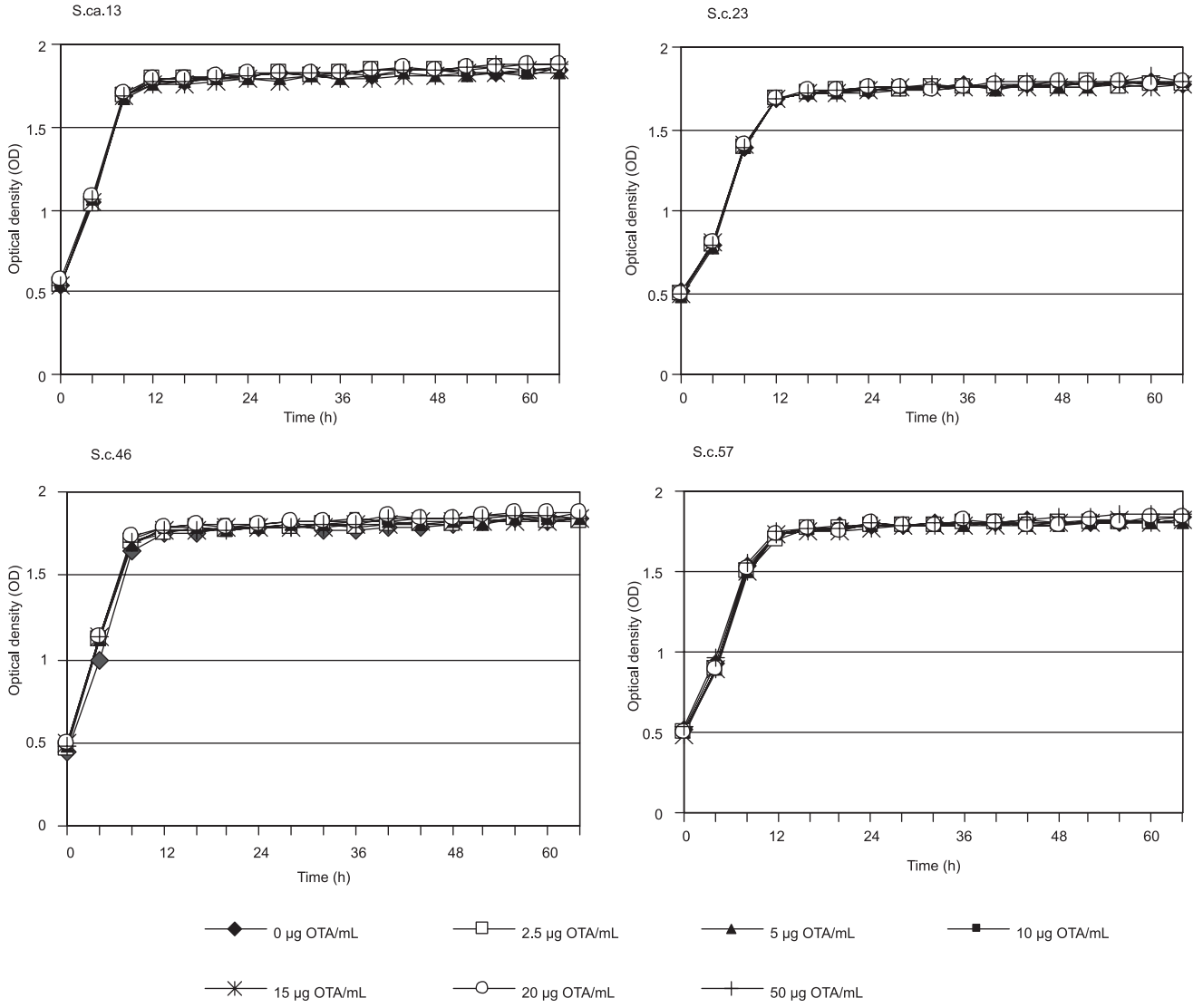

FIGURE 3. Growth curves of brewing yeasts in YEPG medium contaminated with OTA.

The literature includes, for example, mathematical models describing the adsorption of ZEN by the cell wall of the yeast *S. cerevisiae* in vitro [Yiannikouris *et al*., 2003].

The impact of toxin depends not only on the structure of cell membrane (which can differ even within the same yeast species) but also on its integrity (which is influenced by a number of factors). It has been reported, for instance, that in the absence of ethanol, T-2 toxin (2 μg/mL) caused minor reduction in the growth of *S. carlsbergensis*. In the presence of ethanol (5%), 2 μg T-2 toxin per mL caused complete cessation of growth [Schappert & Khachatourians, 1984].

In the study presented in this paper the brewing yeasts were exposed to a toxin-containing medium for a much longer period compared to investigations reported in the literature (where yeast growth was generally examined after several hours of cultivation). In this way it was possible to demonstrate that the yeasts have the ability to resist the inhibiting effect of a toxin as strong as DAS and achieve a biomass yield similar to the one in the control sample. The behaviour of the strain S.c. 57 is a good case in point. Furthermore, the particularly sensitive strains, S.ca. 13 and S.c. 46, began to proliferate in the presence of high ZEA doses (50 and 100 μg/mL) after 30 to 50 h, probably owing to the activation of the resistance system. Some of the fungi and yeast genera have a mechanism of toxin biotransformation into compounds of a lower harmfulness [Böswald *et al*., 1995; Kamimura, 1986]. Zearalenone is transformed, e.g., into two isomers: α-zearalenol (0.5%) and β-zearalenol (95%). Of these, α-zearalenol shows a higher toxicity. T-2 toxin can be transformed into compounds that display traces of toxicity: HT-2 and T-2 triol [Boeira *et al*., 2002; Whitehead & Flanningan, 1989].

In the study reported on in this paper, the effect of the mycotoxins DAS, ZEA and OTA on the brewing yeast strains was examined under model conditions with the use of a synthetic medium and constant growth parameters (oxygenation, temperature) in order to eliminate any factors that might affect the kinetics of yeast growth. A further stage of the study is planned involving mycotoxin-contaminated brewing wort. The research aims at answering the question of how the reduced growth rate and the duration of the log phase that were tested in the model medium will affect the fermentation of contaminated worts.

TABLE 2. Duration (h) of the log phase of brewing yeast growth in the YEPG medium contaminated with DAS, ZEA and OTA.

Toxin	Toxin concentration (μg/mL)	Yeast strain			
		S.ca.13	S.c.23	S.c.46	S.c.57
Control	0	8	10	8	10
DAS	2.5	12	11	12	13
	5	29	13	26	17
	10	48	15	43	21
	15	58	15	60	24
	20	17	18	19	28
ZEA	2.5	9	10	9	9
	5	8	10	8	9
	10	9	10	8	10
	15	9	10	9	9
	20	10	10	9	10
	50	18	16	21	18
	100	20	18	7	20
OTA	2.5	9	11	8	10
	5	9	11	8	10
	10	8	11	8	10
	15	9	11	8	11
	20	9	11	8	10
	50	9	11	8	11

TABLE 3. Biomass yield (ΔOD) of brewing yeasts in the YEPG medium contaminated with DAS, ZEA and OTA.

Toxin	Toxin concentration (μg/mL)	Yeast strain			
		S.ca.13	S.c.23	S.c.46	S.c.57
Control	0	1.36	1.32	1.43	1.33
DAS	2.5	1.37	1.29	1.37	1.36
	5	1.31	1.27	1.39	1.32
	10	1.21	1.28	1.19	1.35
	15	1.07	1.25	1.10	1.31
	20	0.27	1.28	0.30	1.23
ZEA	2.5	1.30	1.32	1.37	1.33
	5	1.34	1.32	1.38	1.31
	10	1.32	1.32	1.37	1.31
	15	1.35	1.30	1.35	1.29
	20	1.33	1.34	1.36	1.30
	50	0.79	1.31	1.19	1.24
	100	0.75	1.21	0.66	1.17
OTA	2.5	1.37	1.33	1.39	1.36
	5	1.35	1.33	1.39	1.35
	10	1.37	1.32	1.40	1.36
	15	1.36	1.31	1.36	1.34
	20	1.37	1.33	1.40	1.35
	50	1.40	1.36	1.41	1.36

CONCLUSIONS

The obtained results indicated that each of the toxins influenced the growth of the brewing yeasts in a different way. Regardless of its concentration in medium, ochratoxin A exerted a poor effect on the growth parameters of yeasts. The influence of zearalenone on biomass growth was found to be disadvantageous only when the highest doses, 50 and 100 μg/mL, were applied. The influence of diacetoxyscirpenol was the strongest; the increase of DAS concentration in the culture medium was parallelled by inhibition of growth of all the yeast strains studied.

Each of the brewing yeast strains developed a specific response to the influence of particular toxins. S.ca. 13 and S.c. 46 lost their specific growth rate when DAS was present in the medium or when the highest ZEA doses were applied. As a result, duration of the log phase was extended in the samples receiving DAS doses higher than 10 μg/mL, and the biomass yield decreased notably. The other two strains, S.c. 23 and S.c. 57, were found to be less sensitive to the toxins studied; their specific growth rates decreased only slightly in the presence of DAS and ZEA. The final biomass yield was comparable to that of the control samples.

On the basis of the results obtained, the following concentrations were chosen for our further investigations which involved fermentation media (brewing wort): 5 and 15 μg DAS /mL; 5 and 50 μg ZEA /mL and 15 μg OTA /mL.

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WPŁYW MYKOTOKSYN DAS, ZEA I OTA NA WZROST DROŻDŻY PIWOWARSKICH

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Celem pracy było określenie wpływu zróżnicowanych stężeń diacetoksyscirpenolu (DAS), zearalenonu (ZEA) i ochratoksyny A (OTA) na wzrost 4 szczepów drożdży piwowarskich w podłożu modelowym YEPG. Badano właściwą szybkość wzrostu, długość fazy logarytmicznego wzrostu oraz plon biomasy. Stwierdzono, że OTA w zakresie stężeń od 2,5 do 50 μg/mL nie ograniczała wzrostu badanych szczepów drożdży. Zearalenon wpływał niekorzystnie dopiero przy dwóch najwyższych stężeniach (50 i 100 μg/mL). Największą toksyczność wykazał DAS, który hamował wzrost wszystkich drożdży począwszy od najniższej dawki 2,5 μg/mL. Badane szczepy drożdży charakteryzowały się indywidualną wrażliwością na toksyny. Najmniej oporne, zwłaszcza na toksynę DAS, były szczepy: *S. carlsbergensis 13* oraz *S. cerevisiae 46.*